Proceedings

of the

Society

for

Experimental Biology and Medicine

Vol. 36. February, 1937. No. 1.

SECTION MEETINGS

CLEVELAND	
Western Reserve University	January, 1937
ILLINOIS	
University of Illinois Medical School	February 9, 1937
MINNESOTA	
University of Minnesota	February 17, 1937
MISSOURI	
Washington University Medical School	February 10, 1937
PACIFIC COAST	
Stanford University Medical School	February 3, 1937
WESTERN NEW YORK	
University of Rochester Medical School	February 13, 1937

9093

Dynamics of Fibrinolysin-Production by Streptococci.*

R. R. Madison and Jeannette D. Taranik. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

To estimate the amount (or potency) of the fibrinolytic "enzyme" formed or secreted by *Streptococcus hemolyticus*, the following

* Work supported in part by the Eli Lilly and Co. Streptococcus Research Fellowship of Stanford University and in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

titrative method has been adopted by our laboratory. Standard solutions of fibrinogen and thrombin are prepared from freshly drawn human blood by the method of Tillett and Garner.[1] Serial dilutions (1:2) are made of the broth culture to be tested. To 0.5 cc. of each dilution there is added 1 cc. of the standard solution of fibrinogen. Coagulation is then brought about by the addition of 0.1 cc. of standard solution of thrombin, the fibrinous clot usually forming within 30 seconds, after which each tube is placed in a thermostatic waterbath (37°C.). Readings are usually made at the end of 15 minutes, 30 minutes, 60 minutes and 2 hours. The highest serial dilution of the broth culture causing complete liquefaction of the clot by the end of 2 hours' incubation is assumed to contain one arbitrary fibrinolytic unit. From this dilution the number of lytic units per cc. of broth culture is readily calculated.

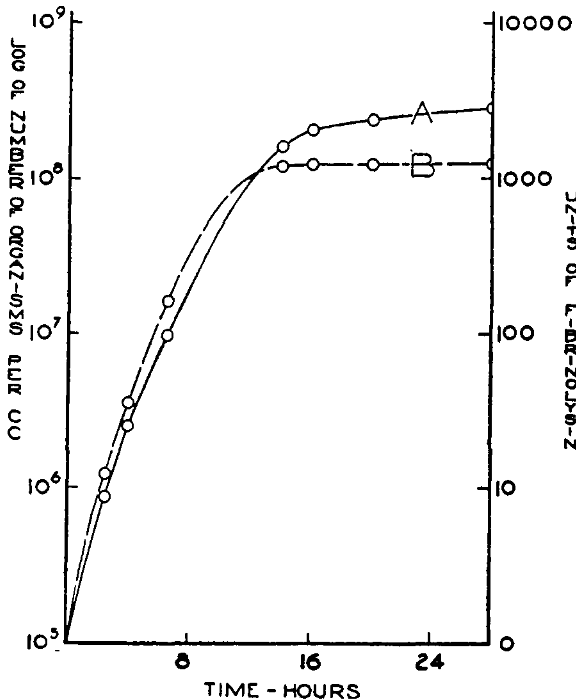

FIG. 1.

Relation of fibrinolytic titer to proliferation-rate. 100 cc. veal-infusion dextrose-broth plus 0.1 cc. 24-hour broth culture of *S. hemolyticus;* incubated at 37°C with constant stirring.

A, Increase in total population per cc. as determined by Petroff-Hausser counting chamber.

B, Parallel changes in fibrinolytic titer per 0.5 cc., plotted as a logarithmic function.

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

Using this method, the quantitative laws of fibrinolysin-production have been determined for numerous strains of *S. hemolyticus*. Typical data thus obtained are recorded in Fig. 1.

From this curve it is evident that the rate of fibrinolysin-production and the rate of test-tube proliferation of streptococci are parallel during the logarithmic phase of population-increase, an apparent quantitative linkage between enzyme secretion and cell-division.

This apparent linkage, however, is not operative beyond the logarithmic phase of population-increase. While with certain strains the fibrinolytic titer remains constant or even increases slightly during the subsequent static phase of test-tube growth, a fairly rapid destruction (or inactivation) of the lytic factor takes place in most older cultures. Three typical sets of data are recorded in Fig. 2.

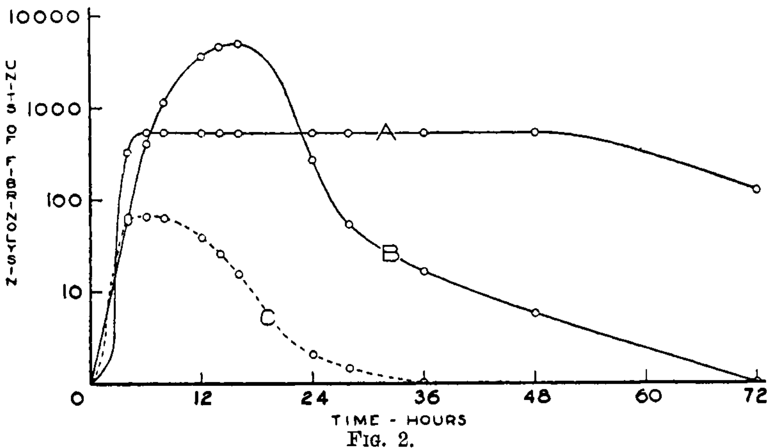

FIG. 2.

Destruction of fibrinolysin in older cultures. 100 cc. veal-infusion dextrose-broth plus 0.1 cc. 24-hour broth culture of *S. hemolyticus;* incubated at 37.5°C with constant stirring.

A, Changes in fibrinolytic titer per 0.5 cc. in Streptococcus Strain No. 211.
B, Changes in fibrinolytic titer per 0.5 cc. in Streptococcus Strain No. 291
C, Changes in fibrinolytic titer per 0.5 cc. in Streptococcus Strain No. 189

From these data it is evident that a routine clinical test of 18- to 24-hour broth cultures of *S. hemolyticus* may lead to erroneous conclusions as to their fibrinolytic (or invasive) properties. A routine test of younger (*e. g.*, 12-hour) broth cultures would presumably lead to greater clinical accuracy.